Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils[1]

J. P. E. ANDERSON AND K. H. DOMSCH
Institut für Bodenbiologie, Forschungsanstalt für Landwirtschaft, D 3300 Braunschweig, Bundesallee 50, Federal Republic of Germany

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A technique using selective inhibitors was used to estimate the relative contributions of bacterial and fungal populations to the respiration of six soils and one litter sample. The ratios of bacterial to fungal respiration in the four agricultural soils, given in percentage of the total microbial activity, ranged from 10/90 to 35/65, with the average ratio being about 30/70. In the forest soils, the ratios were 20/80 and 30/70, and in a beech litter sample, the ratio was 40/60. The fungi clearly dominated in all samples. The ratios were not found to be pH related. The difficulties which had previously limited the use of selective inhibitors for *in situ* soil ecological investigations, such as insufficient inhibitor specificity, inhibitor inactivation or degradation, and errors of measurement caused by elimination of competitor populations, were either resolved or methodologically avoided in the experiments. Inhibitor selectivity was demonstrated using both mixed and pure cultures of microorganisms from each soil. Through the use of experiments with short incubation periods (6–8 h), problems with population shifts and inhibitor degradation were eliminated.

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Nous avons employé une technique d'inhibiteurs sélectifs pour évaluer la contribution relative des populations bactériennes et fongiques à la respiration de six sols et d'un échantillon de litière. Le rapport de la respiration bactérienne sur la respiration fongique dans quatre sols agricoles, en terme de poucentage de l'activité microbienne totale, varie de 10/90 à 35/65, avec un rapport moyen calculé de 30/70. Dans les sols forestiers, les rapports sont de 20/80 à 30/70, et dans une litière de hêtres, le rapport est de 40/60. Les champignons dominent nettement dans tous les échantillons. Les rapports ne sont pas en relation du pH. Les difficultés qui jusqu'à présent limitaient l'usage d'inhibiteurs sélectifs pour les investigations écologiques du sol *in situ*, tel que manque de spécificité de l'inhibiteur, dégradation ou inactivation de l'inhibiteur, les erreurs de mesure causées par l'élimination de populations compétitives, furent résolus ou évités méthodologiquement dans nos expériences. Nous avons démontré la sélectivité des inhibiteurs en utilisant des cultures mixtes et des cultures pures de microorganismes pour chaque sol. En utilisant des expériences avec un court temps d'incubation (6 à 8 h), nous avons éliminé les problèmes des changements de population et de dégradation de l'inhibiteur.

[Traduit par le journal]

Introduction

The combined metabolism of mixed populations of microorganisms in complex substrates such as soils, natural surface waters, or decaying organic materials can be quantitatively determined by measurement of total respiration. Determination of the contributions of individual components of the population to the total metabolism, however, has remained difficult because of the methodological problems involved with simplification of a complex system without destruction of the system itself. Of the techniques thus far developed to examine manyphased ecosystems, the use of isotopes (Brock and Brock 1966; Waid *et al.* 1971), selective inhibitors (Ivarson and Sowden 1959; Domsch 1968; Anderson and Domsch 1973, 1974), analyses for the presence and quantity of products associated with or unique to microorganisms (Miller and Casida 1970; Swift 1973), and either direct determination (Jones and Mollison 1948; Babiuk and Paul 1970; Shields *et al.* 1973) or estimation of individual biomasses (Clark 1967; Clark and Paul 1970; Satchell 1971), followed by calculation of relative metabolic activities have received the greatest attention.

Among the methods mentioned above, only the technique described by Anderson and

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TABLE 1

Soil characteristics

Particle size, %	Soils[a]					
	I	II	III	IV	V	VI_h
2000–630 μ[b]	0.2	5.4	5.1	0.4	2.2	2.2
630–200 μ	37.3	35.3	22.3	1.4	42.8	22.0
200–63 μ	36.6	33.3	15.0	2.4	34.8	15.9
63–20 μ	10.6	13.2	13.4	48.0	7.9	10.7
20–6.3 μ	3.5	5.2	8.4	21.7	3.1	6.3
6.3–2 μ	3.6	3.1	7.3	6.0	2.2	10.3
< 2 μ	8.3	4.5	28.5	20.1	7.0	32.6
pH (KCl)	5.2	6.7	5.5	7.5	4.2	2.9
C_{total}(%)	0.614	1.248	3.785	2.331	3.764	5.605
N_{total}(%)	0.049	0.102	0.399	0.202	0.259	0.528
C/N	10.3	13.3	9.7	10.6	14.2	41.0
MWC[c]	36.0	33.6	51.3	54.8	52.7	210.3
% MWC[d]	39	41.5	46.0	38.0	44.5	47.5
Date collected	Oct.'73	Dec.'73	Aug.'72	Aug.'73	May'73	July'73

[a]Collection sites given in text: I–IV are agricultural soils; V–VI are forest soils. I and III are Braunerde types, IV is a chernozem.
[b]μ, micron.
[c]MWC = maximum water capacity = g H_2O which can be held by 100 g (oven dry wt.) of soil.
[d]%MWC = H_2O content of soil during experiments in % of quantity defined by MWC.

Domsch (1973) allows the contributions of bacterial and fungal populations to soil metabolism to be directly measured. To make measurements, the overall metabolism of the active microflora of sieved soil is temporarily intensified by mixing samples with a readily degradable substrate. Simultaneously, subsamples of soil are also amended with inhibitors which are highly specific for either bacteria or fungi. After a very brief period of incubation, the percentage of contributions of the bacterial and the fungal populations to the soil respiration can be measured by comparison of the respiration of the inhibitor-treated to the inhibitor-free soils. In the soil used for development of the method, the ratio of bacterial to fungal respiration was found to be about 20/80.

The substrate used in the method, chosen because most known soil bacteria and fungi can use it as a source for energy and growth, is glucose. The inhibitors used, which were chosen because of their selectivity at very high concentrations, are streptomycin and actidione. The duration of experiments from which ratios are calculated is limited to a maximum of 6–8 h to ensure that only the activities of the initially metabolically active populations are being measured.

In the present study, the general applicability of the method described by Anderson and Domsch (1973), for use with soils, was investigated. For this purpose, four agricultural and two forest soils, as well as one litter sample, were tested. Of particular interest were the relative contributions of the bacteria and fungi to the basal respiration of these soils.

Materials and Methods

A. Soils

The soils used are characterized in Table 1. Samples of agricultural soils were collected from the upper 10 cm of fields in the vicinity of Göttingen (I), Berlin (II), München (III), and Jerxheim (which is near Braunschweig) (IV). Forest soils were collected from Rieseberg (which is near Braunschweig) (V), a Querceto-Carpinetum forest, and Solling (VI), a beech forest (German IBP site). With the exception of sample III, all soils were collected, passed through a 2-mm sieve, and used immediately in the experiments. Sample III, which had been frozen at −18°C for 1 year, was thawed at 22°C for 24 h before it was sieved. The litter (VI_l) and humus (VI_h) layers of the Solling sample were collected separately. The litter was chopped in a blender, passed through a 2-mm sieve, and then handled like a soil sample.

B. Inhibitors

The inhibitors used were streptomycin sulfate (Bayer) and actidione (= cycloheximide: Upjohn Company). Both compounds were ground in a mortar with talcum before mixing into soil (0.5 g talcum/100 g dry weight soil). Concentrations of inhibitors were calculated using the wet weights of culture medium or the dry weights of soil.

TABLE 2

Growth of microbial colonies from soil dilutions mixed with malt agar containing antibiotics. Inhibitor concentrations shown were the highest at which the selectivity of streptomycin for bacteria or actidione for fungi were maintained

		Inhibitor				
		None	Streptomycin		Actidione	
Soil	Organisms[a]	No. colonies[b]	ppm	No. colonies	ppm	No. colonies
I	Fungi	14.2±3.7	2000	16.3±3.1	2000	3.5±1.5
	Bacteria	64.8±16.4	"	0.7	"	47.7±8.6
	Actinomycetes	5.2±1.1	"	0.5	"	3.0±0.8
II	Fungi	15.3±10.2	1000[c]	19.4±4.0	2000	3.3±2.5
	Bacteria	114.1±12.2	"	4.2±1.2	"	97.7±6.1
	Actinomycetes	5.8±1.7	"	0	"	4.9±1.7
III	Fungi	35.5±6.8	2000	48.8±6.7	4000[c]	29.8±3.3
	Bacteria	210.6±19.5	"	7.0±4.3	"	214.7±17.9
	Actinomycetes	5.0±2.5	"	0	"	3.1±1.2
IV	Fungi	16.4±2.9	2000	26.4±2.1	3000	3.2±1.6
	Bacteria	157.8±14.6	"	3.2±2.7	"	113.6±16.5
	Actinomycetes	9.8±0	"	0	"	5.8±1.9
V	Fungi	11.5±5.4	2000	9.5±4.8	3000	2.0±2.0
	Bacteria	141.7±19.7	"	4.3±2.0	"	149.5±15.2
	Actinomycetes	5.0±3.0	"	0	"	5.3±2.0
VI_h	Fungi	17.5±6.5	1000[c]	14.2±4.1	2000[c]	1
	Bacteria	33.8±3.4	"	4.8±3.2	"	39.2±4.3
	Actinomycetes	0.4	"	0.8	"	0.4

[a]Fungi counted from $1:2\times10^{-5}$ dilutions and bacteria plus actinomycetes at $1:2\times10^{-6}$.
[b]Results are average ± standard deviation of five replicates.
[c]Highest concentrations tested.

C. Organisms

Fungi were isolated from washed particles of soil, and population analyses were made, as described by Gams and Domsch (1967).

D. Measurement of CO_2 Production

The CO_2 produced by pure cultures of fungi in soil, or by soils containing natural microbial populations, was measured on an "Ultragas 3" CO_2 analyzer (Wösthoff Company, Bochum) as described by Anderson and Domsch (1973).

E. Tests for Inhibitor Selectivity

Selectivity tests with streptomycin and actidione were conducted using both mixed microbial populations and pure cultures of fungi isolated from the various soils.

For mixed culture tests, soil-dilution plates were poured containing malt agar plus 250, 500, 1000, or 2000 ppm streptomycin or 500, 1000, 2000, or 4000 ppm actidione. Control plates contained diluted soil plus inhibitor-free agar. Plates were incubated at 22°C for 5 or 14 days after which colonies of bacteria ($1:2\times10^{-6}$ soil dilutions) and fungi ($1:2\times10^{-5}$ soil dilutions) were counted. Five replicate plates were prepared for each treatment.

The effects of the inhibitors on the linear growth of pure cultures of fungi on agar, or their production of CO_2 in soil were tested as described by Anderson and Domsch (1973).

Results

A. Inhibitor Selectivity

Selectivity tests using mixed cultures of microorganisms proved that soil bacterial and actinomycete populations were quite insensitive to actidione, and soil fungal populations were quite insensitive to streptomycin (Table 2). The first significant reduction in numbers of bacterial colonies developing on actidione-treated agar occurred at a concentration of 2000 ppm with soils I, II, and VI_h, and at a concentration of 4000 ppm with soils IV and V. No reductions were noted in soil III, even at the highest concentration tested, 4000 ppm. The fungal populations of all soils were found to be equally insensitive to high concentrations of streptomycin. At concentrations of 1000 or 2000 ppm, reductions in fungal colony numbers were not observed.

In contrast to the resistance of non-target populations to the inhibitors, target populations were found to be quite sensitive. At the lowest

treatment levels tested, which were 250 ppm for streptomycin and 500 ppm for actidione, bacterial and fungal colonies were, with the exception of the fungi in soil III, nearly eliminated from the soil-dilution plates. When the inhibitor concentrations were increased to the high levels shown in Table 2, further reductions of target-colony numbers did not result.

Small numbers of streptomycin-resistant bacteria and actidione-resistant fungi were encountered in all soils. In general, resistant colonies developed very slowly and remained quite small. In soil III, in which an extreme case of fungal resistance to actidione was encountered (Table 2), a single fungus, *Penicillium lilacinum*, was found to constitute 87% of the population surviving on plates treated with 4000 ppm of the inhibitor. In control plates, this fungus was encountered as 15 of a total of 168 colonies. In the population analyses of the fungi isolated from washed soil particles, a method which is selective for fungi existing in the soil in the mycelial stage, *P. lilacinum* was found to constitute less than 0.1% of the population of all soils. It was therefore not included in the population list shown in Table 3.

The resistance of *P. lilacinum* to actidione did not measurably interfere with the experiments used for quantification of the bacterial and fungal contributions to soil respiration. The respiration curves plotted from the samples of soil III which had been simultaneously supplemented with glucose, streptomycin, and actidione (Fig. 1, III, curve D) had slopes which were flat, and this indicated that measurable quantities of rapidly developing, actidione-resistant biomass were not present during the period selected for calculations.

Results of the selectivity tests which were conducted to determine the effects of streptomycin and actidione on the radial growth, or on the CO_2 production of pure cultures of fungi are given in Table 3. The predominant fungi in each of the soils, as determined from the population analyses shown in Table 3, were tested in these experiments. The general insensitivity of these fungi to streptomycin, and their high degree of susceptibility to actidione, are clearly demonstrated. With one exception, the addition of actidione to glucose-supplemented pure cultures resulted in the near total or complete inhibition of CO_2 production. In contrast to this, slight or no inhibition of fungal respiration or growth was caused by the addition of streptomycin.

B. Glucose and Inhibitor Concentrations for Respiration Experiments

The glucose and inhibitor concentrations selected for each soil were determined in a stepwise sequence of experiments as described by Anderson and Domsch (1973). Glucose concentrations were selected so that the CO_2 production curves of inhibitor-free soils increased steadily for at least 6–8 h. Inhibitor concentrations were balanced so that the sum of the effects of the individually applied inhibitors equalled the effect when the inhibitors were added as a mixture. The concentrations selected for samples I through VI_h were (in ppm) I, glucose (G) = 500, streptomycin (S) = 1000, actidione (A) = 500; II, G = 1000, S = 500, A = 1000; III, G = 1000, S = 1000, A = 3000; IV, G = 1000, S = 1000, A = 3000; V, G = 4000, S = 1000, A = 1000; VI_l, G = 8000, S = 3000, A = 4000; VI_h, G = 1000, S = 1000, A = 2000.

C. Bacterial and Fungal Contributions to Respiration of the Soils

The curves used for estimation of the ratios of bacterial to fungal respiration in the soils are given in Fig. 1. Each set of curves was prepared from the averages of four replicate experiments. The CO_2 production of soil samples supplemented with glucose is shown by the curves labeled A; those labeled B show the respiration of the soils which had been mixed with glucose and streptomycin; those labeled C show respiration of samples mixed with glucose and actidione; and those labeled D show respiration after mixture with glucose, streptomycin, and actidione.

Addition of the antibiotics as a mixture to the soils did not result in 100% reduction of soil respiration. This was not expected since the mode of action of both chemicals is the inhibition of protein synthesis. The activity of the glucose-metabolizing systems present in the soils at time of treatment would not be blocked by these inhibitors and would in all probability be capable of degrading the substrate for as long as it were available. Hence, the respiration shown in the curves labeled "D" in Fig. 1 was attributable to biomass existing in the soil before introduction of glucose which was not immediately or directly susceptible to the mixture of strepto-

TABLE 3

Influence of streptomycin (1000 ppm) and actidione (2000 ppm) on the growth and respiration of fungi isolated from four agricultural (I–IV) and two forest (V–VI_h) soils

Fungus	% of total population in soil						% Inhibition: Streptomycin		% Inhibition: Actidione	
	I	II	III	IV	V	VI_h	CO_2[a]	G[b]	CO_2	G
Aspergillus fumigatus	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	0	—	100	—
Aureobasidium bolleyi[c]	1.9	0.8	3.2	3.0	≤0.1	≤0.1	6	5	100	100
Chrysosporium pannorum	≤0.1	3.1	0.4	1.4	≤0.1	≤0.1	0	4.5	100	100
Fusarium culmorum	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	0	0	100	100
F. equiseti	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	0	2	100	100
F. sambucinum[c]	2.4	0.4	4.1	1.4	≤0.1	≤0.1	10	7	55	100
Mortierella alpina	0.4	0.4	1.5	3.1	0.2	0.2	8	10.5	100	100
M. elongata[c]	22.7	3.3	22.5	1.0	1.7	5.4	13	14	100	100
M. hyalina	1.8	1.2	4.4	0.7	6.3	7.9	—	13	—	100
M. nana	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	7	14	100	100
M. spinosa	0.1	≤0.1	≤0.1	1.0	11.4	≤0.1	—	6	—	100
M. zonata	1.8	3.1	6.1	4.8	7.0	4.7	9	9	100	100
Mucor hiemalis[c]	10.3	0.6	2.8	2.8	18.2	0.1	8	0	100	100
Paecilomyces carneus	≤0.1	1.0	12.7	0.1	≤0.1	≤0.1	0	0	100	100
Penicillium janthinellum	≤0.1	≤0.1	6.1	≤0.1	0.8	41.5	0	0	98	100
P. nigricans	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	0	16	100	36
P. herquei	9.6	0.2	0.6	≤0.1	0.2	≤0.1	0	—	80	—
Phialophora cyclaminis B	0.6	1.0	0.8	1.7	≤0.1	≤0.1	—	12	—	100
Phoma eupyrena[c]	2.3	3.5	0.6	4.2	≤0.1	≤0.1	6	5	100	100
Trichoderma hamatum	4.1	2.4	1.5	5.2	14.6	13.6	0	0	100	100
T. harzianum	2.4	4.1	0.2	≤0.1	0.4	9.5	0	0	100	100
T. koningii	2.3	2.2	3.0	0.7	22.4	8.7	0	7	94	100
Verticillium nigrescens[c]	≤0.1	4.3	0.2	6.2	≤0.1	≤0.1	3	16	100	100

[a] CO_2 evolved in % of inhibitor-free control: calculations continued until peak maximum of control had been reached (8–16 h).
[b] G = growth on inhibitor-treated malt agar in % of that on inhibitor-free agar: results are average from five replicates.
[c] For completeness of the population analysis, results with these fungi, which have been presented elsewhere (Anderson and Domsch 1973), are included here.

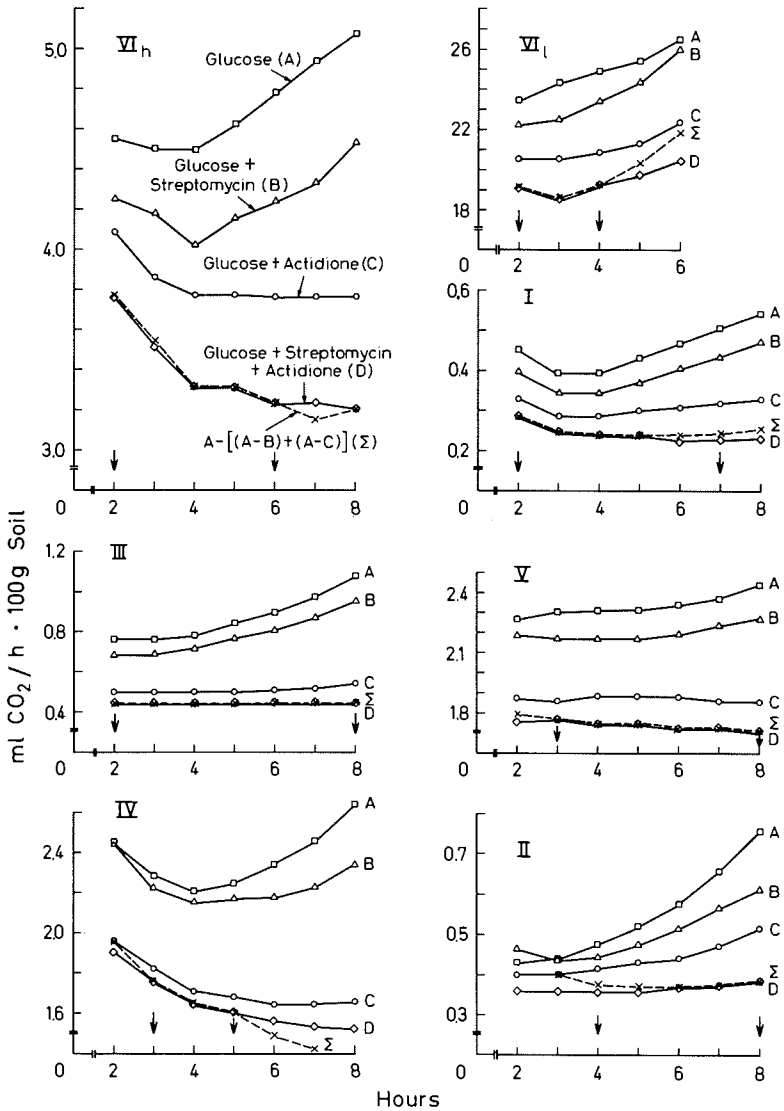

FIG. 1. CO_2 production in soils supplemented with glucose, or glucose plus inhibitor(s). (The soils I–VI_h are characterized in Table 1: VI_l was a beech forest litter sample.) Percentage bacterial respiration = 100 (A–B)/ (A–D); fungal respiration = 100 (A–C)/(A–D). The time intervals between the arrows were used to calculate ratios.

mycin plus actidione. The areas used for calculation of bacterial to fungal respiration in each soil are defined by A–D. This area represents the respiration of biomass formed after, and in response to, addition of substrate. In calculation of initial bacterial to fungal respiration ratios in the soils, the areas defined by A–B and A–C, which when added together equal A–D, are taken to be directly in proportion to the activity of the preformed and selectively uninhibitable bacterial and fungal biomass defined by "D." It is assumed in calculation of ratios that the initial rates of biosynthesis of bacteria and fungi in soils are not extremely different, and that the newly formed bacterial and fungal biomasses are not capable of an extremely different rate of metabolism than the biomasses from which they were formed. The assumption of similar initial growth rates is supported by the data in Fig. 1. These show that the respiration ratios established

at the beginning of the experiments remained constant for at least 4 to 6 h. Should extreme shifts in the initial growth rates of bacterial and fungal populations in soils exist, it would be shown by a very rapid and most probably unidirectional change in A–B/A–C. This did not occur with any of the soils during the time intervals shown in Fig. 1.

Based on the above data, the percentage of respiration of the bacterial populations could be calculated using 100 (A–B/A–D), and the fungal respiration could be calculated using 100 (A–C/A–D). The data obtained with these expressions, which were calculated for each hour of the experiment, were considered valid only as long as $A - [(A-B) + (A-C)]$ (shown in Fig. 1 as curve $\sum$) $= D \pm 5\%$ D, and as long as A–B/A–C did not show steady and unidirectional increases or decreases. Inequalities in the given equation were used to determine if a loss of inhibitor toxicity had occurred (i.e., $\sum > D \pm 5\%$ D) or if loss of selectivity of one or the other inhibitors had occurred (i.e., $\sum < D \pm 5\%$ D). The value, $\pm 5\%$ D used in the equation was selected as the highest reasonable allowance for experimental errors. With the above criteria as limits, the number of hourly measurements used for calculation of averages was defined. The bacterial to fungal respiration in the soils, rounded to the nearest 5%, was found to be: soil I = 30/70; II = 35/65; III = 20/80; IV = 10/90; V = 20/80; VI_l = 30/70; VI_h = 40/60. The fungi were clearly dominant in the litter (VI_l) and in all of the soil samples tested.

Discussion

As pointed out by Gray and Williams (1971), and Parkinson *et al.* (1971), the major difficulties involved with using selective inhibitors to measure the metabolic activity of specific microbial groups in soils are that the chemicals used are often insufficiently specific in action and they are often easily inactivated by soil or degraded by the surviving soil microflora. Further, the use of selective inhibitors causes the removal of competitors. This results in an unnaturally large amount of energy becoming available to the remaining organisms which can then thrive and grow more quickly.

The specificity of the inhibitors used in this and a previous study (Anderson and Domsch 1973) was tested with both pure cultures of soil fungi, and the mixed bacterial and fungal populations from seven different soils. With few exceptions, streptomycin was found to be highly specific as an inhibitor of bacteria, and actidione was highly specific as an inhibitor of fungi. The specificity of streptomycin at high concentrations was also demonstrated by Hine (1962) who found that up to 400 ppm of the compound had little effect on the growth or respiration of the fungi, *Sclerotium rolfsii*, *Rhizoctonia solani*, a *Trichoderma* sp., two varieties of *Fusarium oxysporum*, and two species of *Pythium*. Three other species of *Pythium* were partially inhibited by the compound.

Inactivation of inhibitors in soil can be partially due to their adsorption to soil particles, and partially due to their degradation by the soil microflora. Inactivation of both agents, with a resultant loss of effectiveness, was counteracted in the present method by addition of excess inhibitor to each of the soil samples. Preliminary experiments were therefore conducted with each soil to determine the highest possible concentration which could be added before selectivity was lost. Although degradation of small quantities of both compounds probably occurred in all soils, it was not measurable in terms of either CO_2 production or loss of toxicity, and was not corrected for in any of the experiments.

According to theory, the induction of a partial biological vacuum by removal of competitors should allow the surviving microflora to grow more rapidly than under natural conditions; this, in turn, should result in too high values being registered for the activity of this population under natural conditions. This possible error of measurement was not observed in the short-term experiments conducted here. With most soils, a constant initial rate of development, where A–B/A–C = constant, was established within 2–3 h and then lasted for an additional 3–6 h. This allowed the initial bacterial to fungal respiration ratio to be calculated as an average of three to six measurements.

The results obtained by the use of this method lend support to the hypothesis that the fungal biomass dominates the metabolism of many agricultural and many forest soils. Although the numbers and types of soils thus far tested do not allow broad generalizations to be made, it would seem clear that the degree of fungal dominance is not dependent on the pH of the soil. In the soils tested here, the pH ranged between 2.9 and

7.5. The pH of the soil with the highest percentage of bacterial activity was 2.9, and that with the least bacterial activity was 7.5.

Correlations between the degree of fungal dominance and the carbon or nitrogen content, or the soil-particle-size distribution were also not evident in these experiments.

Of the other methods used for investigation of the activities of individual microbial populations in soils, determination of biomasses, followed by computation of the probable individual activities has most often been used. Results from these measurements have indicated that fungi play a dominant role in many soils.

Satchell (1971), who studied the energy balance in a mixed deciduous forest in England (Meathop Wood, British Woodland Ecosystem Study, IBP Site), estimated that the biomass of the fungi was about 60 times greater than the biomass of the bacteria. He concluded that the fungi were the major decomposers in the forest soil. In the litter and two forest soils investigated in the present work, the fungi clearly dominated the metabolism, although the degree of dominance was much less than that found by Satchell (1971).

The biomass measurements of Shields *et al.* (1973), Parkinson (1973), and Parkinson as cited by Clark and Paul (1970), for a grassland soil (Matador Project, Canadian IBP) indicated that the soil biomass present as bacteria and fungi was dominated by the fungal component. If the quantity of an individual microbial biomass in soil can be roughly equated to the degree of its metabolic activity, the fungi would undoubtably be the major decomposers in these soils. Clark (1967), in an analysis of his own results and data from the literature, estimated that the bacterial biomass of field soils, which is about half that of the fungi, is responsible for about one-fifth of the total soil respiration. In the field soils tested here and in our previous study (Anderson and Domsch 1973), bacteria were responsible, on the average, for about one-third as much respiration as the fungi.

The results presented in this communication have clearly indicated that selective inhibitors can be used as a tool to investigate the metabolic relationships of microbial populations in soil samples.

In view of the data given concerning the selectivity of streptomycin and actidione, it is suggested that the method could be safely and considerably shortened by elimination of the tests for inhibitor selectivity. This, in turn, would eliminate the need for isolation and cultivation of pure cultures of fungi. Also, as long as the duration of experiments is kept short (6–8 h), tests for CO_2 production from degradation of the inhibitors could also be eliminated. The experiments necessary for the method would then be reduced to (*a*) establishment of the substrate concentration (one experiment); (*b*) establishment of inhibitor concentrations (three to five experiments); and (*c*) quantification of bacterial and fungal contribution to soil respiration (four replicate experiments).

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